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Community Structure and Production of the Macrobenthos on Four Artificial Reefs in the Mississippi Sound in Relation to Substrate and Profile Type

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COMMUNITY STRUCTURE AND PRODUCTION OF THE MACROBENTHOS ON FOUR ARTIFICIAL REEFS IN THE MISSISSIPPI SOUND IN RELATION TO SUBSTRATE AND PROFILE TYPE

by

Patrick Daniel Gillam

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ABSTRACT

COMMUNITY STRUCTURE AND PRODUCTION OF THE MACROBENTHOS ON FOUR ARTIFICIAL REEFS IN THE MISSISSIPPI SOUND

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In recent years, the Mississippi Department of Marine Resources (DMR) has made a concerted effort to enhance its coastal fishery resources by creating artificial reefs. Through this effort, 73 nearshore reefs have been created across the MS coast. Different substrate types used to construct MS artificial reefs include oyster shell and cement rubble. Two types of reef architecture used include high profile breakwater and low profile submerged structures. This study compared the benthic biota associated with oyster shell and cement rubble substrates among four artificial reefs representing high and low profile structures in summer 2011. Colonized benthic biota were quantified from four pairs of substrate baskets containing oyster shell or cement rubble that were submersed for six weeks at each reef. Split-plot ANOVA between substrate type (within-subject) and profile type (between-subject) for several functional metrics, including production potential, showed no significant differences. However, species-specific split-plot ANOVAs, unveiled several individual taxa had significantly different production and/or biomass between profile types and/or substrate types not apparent in the cumulative tests. Although some taxa failed the homogeneity of variance assumption, paired substrates for those taxa were pooled and tested using a one-way ANOVA interpreted using Welch's test. This test unveiled several taxa having significant differences in biomass and production between profiles. MDS plots illustrate differences

in ordination space among substrate types and profile types. PERMANOVA found significant differences in community similarity between high and low profile structures. SIMPER further disclosed how the abundances of multiple taxa drove dissimilarity values between reef profile types and substrates.

ACKNOWLEDGMENTS

I would like to thank my graduate advisor, Dr. Chet Rakocinski, as well as my other committee members, Dr. Mark S. Peterson and Ms. Sara LeCroy. Additionally, I want to thank everyone else who has given me support and advice throughout the course of this degree; Kelsey Gillam, my lab mates, and many other faculty, staff, and students, who have aided me through my time at the Gulf Coast Research Lab.

DEDICATION

I would like to dedicate this thesis to my parents, John and Sherry Gillam, who have guided and assisted me through my educational and professional career. I would also like to dedicate this thesis to my wife, Kelsey Gillam, and her family who have personally helped and encouraged me through the course of this degree.

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CHAPTER I – INTRODUCTION

Background

Artificial reefs are man-made structures placed on the sea floor with the primary purpose of increasing the overall carrying capacity of fish and invertebrates for commercial and recreational fishing activities (Woods 1999). This task is often accomplished by increasing habit complexity (Wilding and Sayer, 2002). Locally, artificial reefs tend to be analogous to oyster reefs in terms of increased production and functionality as a refuge and a food source for multiple species (Coen *et al* 1999). Peterson *et al.* (2000) went so far as to call oyster reefs the "temperate zone analog of the tropical coral reef."

The United States and Japan have been two main producers of artificial reefs; however, the policy objectives and designs of these two artificial reef programs differ greatly (Wilson and Van Sickle 1987). The United States deploys reefs with the primary intention of attracting adult fish, whereas Japan aims to improve spawning and recruitment (Bohnsack and Sutherland 1985). Further, artificial reefs are not just limited to the promotion of fisheries. Other applications include 1) to mitigate against habitat loss, 2) to serve as breakwaters, 3) to reduce fishing pressures in other areas, 4) to provide locations for recreational diving, 5) to prevent trawling in certain areas, and 6) to increase the socio-economic benefits of nearby locales (Bohnsack and Sutherland 1985; Baine 2001; Adams *et al.* 2006).

Attraction vs Production

A major historical controversy over artificial reefs surrounds the attraction versus production debate (Pickering and Whitmarsh 1997). If food or shelter are locally limiting

resources for reproduction, then by providing these resources, artificial reefs may increase the local production of some species. However, if artificial reefs only attract resident fish and invertebrates, they will concentrate fish without increasing the total production of biomass, which can lead to potential overfishing of the resident stock (Bohnsack 1991). To properly manage fisheries surrounding artificial reefs, it is important to fully understand their dynamics and their production potential as they pertain to the debate. While the debate mostly concerns fisheries production, less is known about the extent to which these reefs might support primary production and thus promote secondary production. Past experiments have demonstrated successful benthic colonization on hard substrata (Sampaolo and Relini 1994; Foster *et al.* 1994) in addition to enhanced benthic productivity on artificial reefs (Smith *et al.*, 1979; Bohnsack *et al.* 1991; Bombace *et al*. 1994). This increased benthic productivity on artificial reefs may be due to trapping of food and other resources as a result of increased sedimentation, waste products (nutrients), and/or detached epifauna (Foster *et al.* 1994).

Sessile invertebrates and algae on artificial reefs are known to attract fish (Anderson *et al.* 1978; Johnson and Stein 1979; Dudley and Anderson 1982; Wallace and Benke 1984) by serving as valuable food sources (Johnson *et al.* 1994). However, despite evidence for both attraction and production scenarios, much still needs to be learned about how these artificial structures function ecologically. To address the attraction versus production debate, it is essential to measure the production capacity and concurrently characterize the community structure of macrobenthic organisms associated with various forms of artificial reefs.

Reef Materials

Artificial reefs have historically been constructed from a wide variety of materials (Table 1). In the United States, much of the construction material comes from sources of discarded goods (Bohnsack and Sutherland 1985) and often utilizes "materials of opportunity" (Woods 1999). When building reefs, organizations may choose to utilize pre-designed reef materials, such as Reef Balls ™, to create effective habitat.

Table 1

Approved and disapproved materials for use in construction of artificial reefs in Mississippi

Materials Approved by Mississippi	**Materials Disapproved by Mississippi**
Shell	Vehicle Tires
Rock	Wood
Concrete	Automobiles
Railroad Boxcars	Aircraft
Steel-hulled Ships, Boats, and Barges	Fiberglass Boat Hulls and Molds
Mineral Producing Platforms	All Coal, Oil, and Municipal Combustion Byproducts (Except Coal Ash)
Manufactured Materials Using Coal Ash	White Goods (i.e. Refrigerators, Washing Machines, etc.)
Designed Materials	
Military Hardware	

(Woods, 1999).

The National Artificial Reef Plan presents five general selection criteria for reef materials: function, compatibility, stability, durability, and availability (Stone 1985).

Function requires a material that is suitable for performing as an appropriate artificial habitat for target fish and invertebrates (Woods 1999). Reef function takes into account proper interstitial spaces, surface area, vertical elevation, and the stimulation of epiphytic growth. Compatibility refers to how the material reacts with the marine environment to minimize environmental risks. Reefs should be made from stable design to ensure stability, and as such they should not deteriorate over time ensuring durability. Availability revolves around economic practicality (Woods 1999). In the past, discarded tires were often used for reefs due to their affordability and availability (Stone *et al.* 1979), but their use eventually led to environmental consequences. For example, the Osbourne Reef off the coast of Fort Lauderdale was expanded through the use of discarded tires. However, this effort ultimately failed and actually has come to be seen as a natural disaster. Over time, tires both deteriorated and have become dislodged, resulting in tires washing up on shore and causing damage to nearby coral reefs. Both the scale and the complexity of this disaster have actually prohibited removal of the reef, although such a plan of action was written by NOAA in 2009 (http://www.dep.state.fl.us/waste/categories/tires/pages/osborneproject.htm).

As a naturally occurring material, oyster shell has a long history of being used in Mississippi (MS) to enhance commercial oyster production. Concrete is another readily available material that is "environmentally compatible" (Woods 1999. It is often used for designed reef structures or obtained as rubble from razed buildings, parking lots, bridges, etc. In a review by Baine (2001), the most common construction material was concrete (25% of citations), followed by typical rock materials as the second most common source (9% of citations).

Management

While artificial reefs have been used for centuries, resource awareness did not provoke interest in reef management until the 1980s. The National Fishing Enhancement Act of 1984 provided a plan to assist those interested in artificial reef development and management (Woods 1999). The Enhancement Act led to the further development of the National Artificial Reef Plan, which serves as a dynamic plan used for amending new information, and yielding several major revisions since original publication in 1985 (Stone 1985). Improper reef development and construction can lead to an inefficient use of time, funds, and material. Improper reef materials can also harm the marine environment through effects like dangerous chemical exposure due to leaching (Collins *et al.* 2002). Since the implementation of the National Artificial Reef Plan, major reef planning and development has come under the control of individual state plans (Stone 1985). These plans provide guidance and instruction on properly constructing, deploying, and managing artificial reefs to enhance the fish habitat and benefit the fisherman.

The MS Artificial Reef Plan came into fruition in November 1999 well after the first known reefs were constructed in MS in 1960. Efforts to construct offshore reefs between 1972 and 1978 involved the sinking of five World War II Liberty ships. Permits for these are presently held by the MS Gulf Fishing Banks, Inc. (MGFB), including a total of 32 site permits for reefs ranging in material from the aforementioned Liberty hulls to concrete housing units to oyster and clam shell (Woods 1999). The first monitoring and evaluation of the offshore reefs in MS was conducted from 1975-1978 by Lukens (1980). The MS Sea Grant Advisory Service and the MGFB implemented an artificial reef monitoring program focusing on reef material in 1985, with the MGFB continuing the

monitoring of the physical parameters (Woods 1999). In 1988, the Gulf States Marine Fisheries Commission began monitoring the artificial reefs with side scan sonar in order to address reef effectiveness in attracting and maintaining fish population (Lukens and Cirino 1989).

Mississippi Artificial Reefs

Artificial reefs have been placed near the coast of MS with the primary objective of increasing the overall carrying capacity of fish and invertebrates for commercial and recreational fishing activities. The state of MS, along with the MS Department of Marine Resources (MSDMR), has aided this effort with the deployment, maintenance, and enhancement of 73 nearshore reefs throughout the MS Sound (Woods, 1999; http://www.dmr.ms.gov/marine-fisheries/artificial-reef/73-inshore-reefs). Reef designs are generally constructed as either high profile, where the vertical profile of the reef reaches the water's surface, or low profile, where the profile remains fully submerged and close to the bottom. Based on the Artificial Reef Plan and the Artificial Reef Development Plan for the State of MS for reef construction in MS nearshore areas, approved materials include oyster shell, concrete rubble, and limestone rock. Presumably, the choice of reef formation type and material will affect reef complexity, and may potentially limit the kinds of organisms that can thrive on that structure (Bohnsack and Sutherland 1985). The rationale for creating these reefs, in addition to protecting shoreline areas, is to increase the amount of sheltering and foraging area for invertebrates and fish; however, the efficacy in this regard has not been closely examined for artificial reefs within MS Sound.

Recent Studies of Mississippi Artificial Reefs

Several complementary studies of artificial reefs in the MS Sound have been undertaken. Perry *et al.* (2001) examined colonization of invertebrates on nearshore reefs using crates of crushed limestone or oyster shell that were systematically pulled every three months for a seasonal representation of community development. They found higher abundances of invertebrates in summer months due to more recruitment and lower abundances in winter months due to presumed seasonal migrations and increased predation rates. Differences in species abundances between substrate types were species-dependent. A concurrent study by Larsen *et al.* (2001), using the same methods but focusing on vertebrates, showed species composition or size of the individuals was based on refuge availability, with larger individuals inhabiting larger holes in the substrate.

A multifaceted assessment of reef function of high and low profile artificial reefs has recently been done by investigators at the USM Gulf Coast Research Laboratory. Salamone (2012) showed a significant difference among reef types in seasonal marine fungal biofilm communities, and the lack of dominant fungal species. Wilking (2013) found a higher microbial biofilm presence on low profile reefs than high profile reefs in addition to higher abundances of biofouling macrofauna (e.g., barnacles). In other research focusing on primary productivity on the reefs, Mazzei and Biber (2015) showed that artificial reefs do not enhance primary production regardless of reef type. Matten (2013) showed a significant difference in the cryptic fish assemblages between high and low profile reefs; community differences between profile types were mainly driven by *Hypsoblennius hentz*, *Gobiosoma bosc*, *Hypleurochilus* sp., unidentified Blennidae, and *Gobiesox strumosus*, all of which were all relatively more abundant on low profile reefs.

Diets of *Gobiesox strumosus* were significantly larger in volume at low profile reefs than at high profile reefs. Barnes (2014) showed that prey availability may have played a major role in the occurrence of predatory fish and found significant a seasonal difference in diet of Spotted Seatrout, *Cynoscion nebulosus*, near artificial reefs where multiple prey items were available in summer, but were absent in the fall. As an opportunistic forager, the diet of Spotted Seatrout generally consisted of various fish and crustaceans.

Focusing on variability in macrobenthic function and structure among MS nearshore artificial reefs will complement the aforementioned studies by Salamone (2102), Wilking (2013), Mazzei and Biber (2015), and Matten (2013). Benthic communities associated with reefs play an important role in ecosystem structure, function, and energetics by enhancing nutrient cycling and providing energy flow to higher trophic levels via secondary production (Keast and Harker 1977; Covich *et al.* 1999; Vadeboncoeur *et al.* 2002; Vander Zanden *et al.* 2006; Northington *et al.* 2010).

By examining the secondary production on these reefs, I was able to obtain insights into the basic trophic patterns that drive many of these systems. By understanding community structure, future managerial decisions can include information on benthic taxa as indicator of ecosystem health. As such, the objective 1) was to document functional differences in the macrobenthic community structure on four reefs in the MS Sound (two high and two low profile reefs), primarily focused on the secondary production potential for two commonly used substrate types (concrete rubble and oyster shell) in Mississippi. Additionally, potential differences in biomass and organismal density were addressed between basket substrate and reef type, as well as potential differences in the distribution of biomass among different size classes of

organisms as another functional indicator. The objective 2) was to observe differences in the community structure on different reef types and basket substrate type. Finally, the objective 3) was to identify differences in production and biomass for taxa found to be driving differences in community structure.

CHAPTER II – MATERIALS AND METHODS

Sample Sites

Reef sites for this study were selected from a map of 55 artificial reefs (2 high profile reefs, 53 low profile reefs; http://www.dmr.ms.gov/marine-fisheries/artificial-reef/73-inshore-reefs) within the MS Sound obtained from MSDMR. Of these sites, there were only two high profile reefs within nearshore MS waters, and both were identified and selected as suitable high profile reefs for this study: Katrina Key and Square Handkerchief (Figure 1; Table 2). The high profile reefs were constructed of large pieces of reinforced concrete that were taken from local deconstructed bridge remains. The 53 low profile reefs within the MS Sound were constructed primarily of oyster shell. Due to the sedimentary nature of MS Sound, deposited reef material often settles into the mud and may be fully submerged over time. To combat this, MSDMR periodically supplements these artificial reefs with more material. In order to select the most appropriate low profile reefs, five reefs were initially chosen for reconnaissance based on their locations within the MS Sound. These reefs were further assessed for suitability by mapping their footprints, as well as those of the high profile reefs.

Sample Site Mapping

Reef mapping occurred during the winter of 2010 to 2011. The five selected low profile reefs and two high profile reefs were mapped for the dual purpose of confirming the reef suitability and determining the full footprint areas of the reefs. MSDMR provided reef sites and the “reef box”, which gave the maximum possible extent of the reef footprint, but did not delineate actual reef size. Transects were used to obtain the most

accurate assessment of the submerged low profile reefs (Matten 2013). Transects were spaced

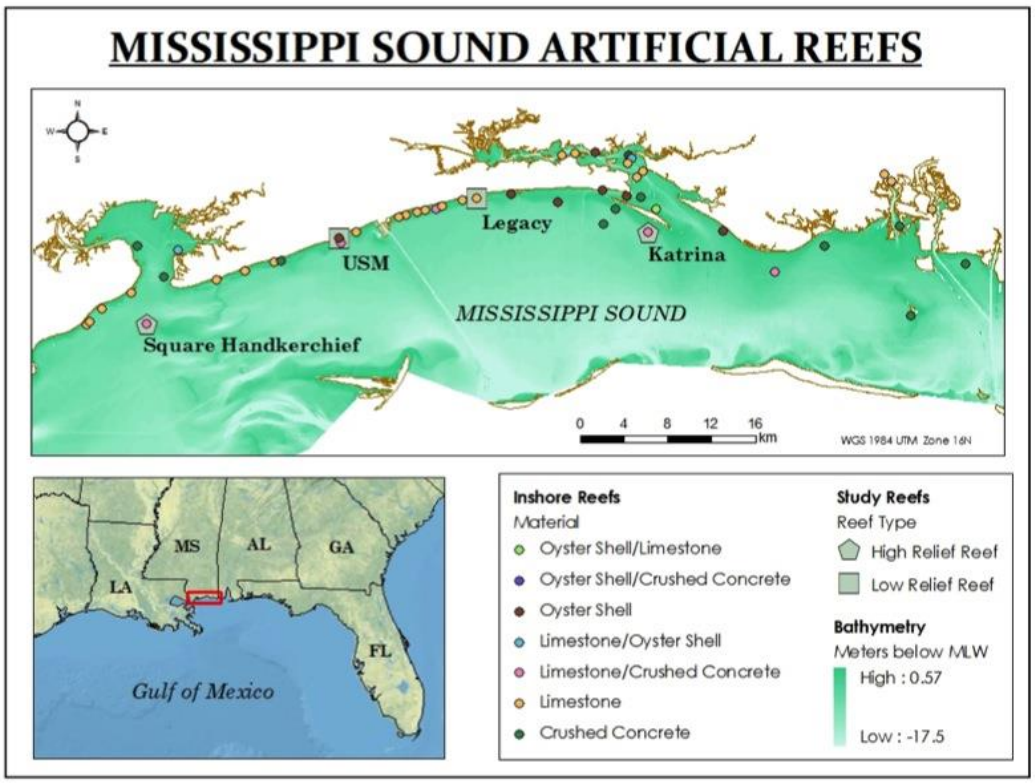

Figure 1. Locations of the four artificial reefs selected for this study, in addition to the construction materials, reef profile types, and general bathymetry of all reefs located in the Mississippi Sound.

(Mazzei and Biber 2015).

about 20 m apart over the entire area of the given "reef box." Within each transect, the bottom substrate was assessed using a PVC pole to measure the bottom hardness and GPS coordinates were recorded. The substrates were categorized as mud/sand, fine material (gravel), coarse material (oyster shell), or solid (reef) based on the poling data. Because the two high profile reefs breached the surface of the water, the visible reef was used as a starting point from which the PVC pole was used to probe the reef substrate,

moving outward from there until the edge of the reef was determined. GPS locations for the outer edge of the reef were recorded at roughly 20 m intervals around the visible portions of the high profile reefs. Spatial reef data were plotted using ArcGIS (ver. 9.3) to generate maps of the reef footprint for all potential sampling sites. The estimated area of the low profile reefs was obtained by interpolation of a raster image using the natural neighbor tool in ArcGIS. This method estimates values at undetermined points on the raster grid based on weighted proximities to known data points. From this interpolated raster map, the total area of each surveyed reef was estimated. The low profile reefs with the greatest estimated total area concentrated in a single block were considered the most suitable for sampling (Matten 2013). These reef sites were chosen based on size, location, and the best scenario for the proper deployment of sampling gear.

Reef Comparisons

The reefs examined in this study represent two reef profile types. High profile reefs are much larger in both area coverage and vertical profile than low profile reefs (Table 2). In addition, construction materials and the topography of the surrounding areas are vastly different for high profile reefs and low profile reefs. Located off of the south side of Deer Island near Biloxi Bay, Katrina Key was constructed from large pieces of reinforced concrete rubble and exposed rebar originating from a destroyed bridge. Katrina Key was deployed parallel to the open side of Deer Island, thus providing an exposed side with higher wave action and a protected side. Located south of Bay St. Louis, Square Handkerchief Key was of similar construction to that of Katrina Key, consisting of large blocks of reinforced concrete originating from a different destroyed bridge. Square Handkerchief Key was constructed in a “U” shape, but due to its

orientation, the direction of the wave action on the reef varies and is dependent on wind direction. Both high profile reefs were placed on mud bottoms. In contrast, both of the low profile reefs were similarly constructed of mostly of oyster shell, and placed on a sandy bottom.

Table 2

General description of the four artificial reefs selected for this study.

Reef Site	Location	GPS Coordinates	Profile	Construction Material	Material Deployed	Depth	Reef Footprint
Katrina	Ocean Springs	30.3546° N 88.8352° W	High	Concrete	2006	3 m	14,566 m^2
Legacy	Biloxi	30.3827° N 89.0033° W	Low	Oyster Cultch	2007	2 m	2,577 m^2
USM	Long Beach	30.34466° N 89.1233° W	Low	Oyster Cultch	1996	2 m	4,039 m^2
Square Handkerchief	Bay St. Louis	30.2710° N 89.3142° W	High	Concrete	2003	3.5 m	11,919 m^2

(Barnes 2014)

Sampling Gear

Cylindrical artificial substrate baskets (27.94 cm in length and 16.51 cm in diameter) constructed of 2.54 cm square PVC coated galvanized wire were deployed for this project. These baskets allowed for the unimpeded movement of the benthic fauna in and out of the basket while allowing direct placement on the reef itself. Pairs of baskets representing both concrete rubble and oyster shell substrata were rigged together at each of four sites per reef. The order of substrate type within each paired basket was determined randomly using a coin flip. Baskets were separated from each other by a 0.5 m line between baskets, and by 1 m from 4.55 kg cement weights at each end of the line (Figure 2). An additional float line extended to the surface of the water.

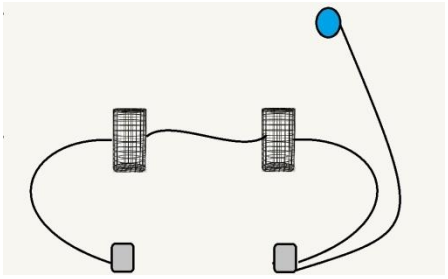

Figure 2. . Basket rig used in sampling, with each basket filled with either concrete rubble or shell connected to two weights with a single buoy line to the surface.

Substrate Selection

Because of their use as common substrate materials in the MS artificial reef program, similar construction materials to both the high profile and low profile reefs, concrete rubble (rock) and oyster shell (shell) were selected for this study. Rock was collected from a processing site whereas shell was obtained from a seafood processing dump site. All baskets were filled with one of the two substrates (16 baskets of rock, 16 baskets of shell).

The surface areas (SAs) of the rocks and shell were estimated to permit normalization of the organism metrics to per m^2 values. The SAs of 20 pieces of rocks and 20 shells spanning the entire range of available sizes were obtained using a NextEngine 3d scanner to obtain conversion relationships of SA relative to linear dimensions and weights of the substrate pieces. The best regressions for estimating SA involved using the weights of individual pieces of rock, weighed using an Ohaus® Defender 5000 scale. Weights of individual shells in addition to the lengths along the central axis were used to estimate SA of the shell. Thus, cumulative SA of material within filled baskets was estimated from weight and/or length of all substrate pieces contained in the baskets.

Deployment of Baskets

Four pairs of baskets were placed on each reef on 9 August 2011 at predetermined sites, as determined from GPS maps of the study areas. Due to the dissimilar construction designs of high and low profile reefs, different gear placement strategies were used to obtain representative coverage of each type of reef sampled. For high profile reefs (Katrina and Square Handkerchief Reefs), basket placement was initially targeted well inside the two opposite ends of the reefs, with the baskets placed on both the north and south sides. The actual placement of the baskets was accomplished by poling the targeted area to ensure suitable substrate, taking into consideration the likelihood of movement, the likelihood of entanglement, stability of the substrate, and ease of retrieval. If the initial targeted area did not meet these criteria, sites within close proximity to the targeted area were evaluated until an appropriate site was found. The paired baskets were slowly

lowered by hand off of the bow of a skiff, with proper care to ensure that the paired baskets were not overlying one another.

Target sites for the low profile reefs (Legacy and USM reefs) were predetermined to be representative, considering the size and topography of the reef. Upon arrival, the targeted sites were poled to ensure the presence of hard reef substrate. If the targeted site was unsuitable, sites were scouted *in situ* within a close proximity of the targeted site until a suitable site was found. Once an appropriate site was found, baskets were lowered using the same method as at the high profile reef, ensuring that the paired baskets were not overlying each other. GPS coordinates, weather conditions, and wind direction were recorded. In addition, wind speed (kph), air temperature (°C), depth (m), secchi depth (m), and surface and bottom measurements for water temperature (°C), dissolved oxygen (mg/L), and salinity (ppt) were measured and recorded at all basket sites for each high profile reef. These metrics were recorded only once at each low profile reef since the close proximity of baskets resulted in identical readings.

Basket Retrieval

Baskets were retrieved by snorkelers and free diving after a deployment time of 6 weeks. The divers first placed a 4 mm mesh bag over each individual basket *in situ* and the bags were then closed using the drawstring in order to prevent the loss of organisms. Each pair of baskets was pulled up gently using the buoy line from the boat, while one person assisted in the water. As each basket breached the surface of the water, it was immediately placed in a large plastic tub. The mesh bags were first removed and rinsed into individual 18.92 L buckets filled with filtered seawater from the site. Baskets were disconnected from the rig and placed into their respective buckets. The plastic tubs,

which had previously held the baskets, were also washed into the buckets, and were then sealed and labeled. The same physical-chemical parameters measured upon deployment were measured and recorded upon retrieval. Two sets of paired baskets from Square Handkerchief reef were excluded from analysis; one pair of baskets had moved far off of the reef and a second pair moved off the reef enough to become buried in mud, and clearly contained low biomass due to burial.

Inshore Sample Processing

All buckets containing the baskets were transported to the USM GCRL upon the completion of retrieval. The contents of each basket were rinsed within the original bucket to remove any loose organisms. Next, all of the contents and associated organisms were carefully placed into a large plastic tub partially filled with filtered seawater. The basket was rewashed into the original bucket to remove remaining organisms. All invertebrates collected from each step were combined such that each sample represented one basket. Each sample was placed into labeled containers partially filled with filtered seawater. Fish were collected in a separate labeled container for a separate study and placed on ice for 15 minutes then preserved with 5% formalin. Each individual substrate piece (rock and shell) was washed into the tub to methodically remove all organisms from the substrate before placing it back into its respective 18.92 L bucket. Water containing organisms was poured from the tub and rinsed through a 0.5 mm sieve. The contents were placed into a labeled jar filled with 5% formalin, and individual buckets containing substrate and seawater were retained in the lab on aeration overnight due to time constraints.

The following day, air stones were removed and buckets containing substrate moved to a processing station where individual pieces of material for each sample were thoroughly washed, scrubbed, and scraped into a plastic tub filled with filtered seawater to ensure that all epifauna were removed. The tub contents were poured through a 0.5 mm sieve and placed into their respective labeled containers filled with 5 % formalin. Next, all of the processed substrate material was placed back into a18.92 L bucket, filled with fresh water, and left to sit for 15 minutes to evacuate the remaining fauna from crevices in the substrate. Each piece of material was lightly rinsed within the fresh water and placed back into the original basket. The contents of the fresh water rinse bucket were poured through a 0.5 mm sieve and combined with its respective sample in the labeled jar containing 5% formalin.

Laboratory Processing

In the laboratory, samples each representing one basket, were stained with Rose Bengal for 48 hours to improve the detection of organisms Following this procedure, samples were subdivided by gentle rinsing through stacked 2.0 mm and 0.5 mm sieves to increase processing efficiency of the coarse and fine fractions. Material that passed through the 0.5 mm sieve was not retained. Material from the 2.0 mm fraction was placed into a labeled vial filled with 5% formalin, and 100% of the retained 2.0 mm fraction was processed, first by sorting organisms into three labeled vials representing major taxonomic groups: Annelida, Mollusca, and Arthropoda. A fourth vial was also used miscellaneous taxa. Smaller organisms found clinging to larger organisms in the 2.0 mm fraction were also quantified. The 0.5 mm fraction was subsampled using a Motoda Plankton Splitter if the sample was considered to be excessively large. The ideal sample

size was determined to be about 12 ml of material for ease and efficiency of sample processing. First, the 0.5 fraction was placed into a graduated cylinder and left to settle for 15 minutes to determine if the amount of material substantially exceeded the target amount (12 ml). If the volume of the material was below or close to the target volume, 100% of the sample material was picked. If the amount of material greatly exceeded the target volume (>12 ml), the sample material was subsampled by evenly distributing it within a Motada Plankton Splitter, and subdividing it consecutively until the target amount (12 ml) was reached. For example, an initial amount of 48 ml of sample material would be split twice to obtain 12 ml of material. The volume of the final sample was measured to enable normalization of abundances to that volume. All splits were recorded to enable later estimation of abundances as a multiple of the number of splits. A total of 70% of the samples were subsampled. The resulting estimated 12 ml of material was completely sorted for organisms and seperated into three labeled vials representing major taxonomic groups (Annelida, Mollusca, and Arthropoda) and a fourth vial for remaining miscellaneous taxa.

Sorted organisms were identified to the lowest practical level and separated into body-size categories (size-taxon fractions) using a series of laminated grids analogous to the series of sieve sizes normally used for production studies: 8.0, 6.0, 4.0, 3.0, 2.0, 1.5, 1, 0.75, and 0.5 mm size fractions. This method was devised by Ferguson and Rakocinski (2008) to emulate sieve sizes used by Edgar (1990). Grids were used as opposed to sieves to enable more accurate size fractionation while preventing unnecessary physical damage to the organisms. Organisms that were larger than 0.31 g were deemed too large for this

study and were not analyzed. The maximum size limitation was provided through size categorizations used in further analysis.

Several approaches were used to estimate mass from body size due to the size and weight limitations of the various methods. Biomass for larger organisms, such as the crabs and shrimp, was estimated from linear measurements of individuals through established regressions of blotted wet weight (g) vs. a prescribed linear size measurement (carapace widths for crabs and carapace length for shrimp; mm) based on subsamples of individuals. Alternatively, volumes of known numbers of smaller organisms within size-taxon fractions were determined using image analysis following Rakocinski and Zapfe (2005). Volumes of organisms were measured via image analysis using MetaVue Version 7.1.7.0 software and a Nikon SMZ 1500 camera. Organisms were placed onto a calibrated squash-plate made from microscope slides and spread into a blotch of uniform thickness. The perimeter of the saved blotch-image was traced twice to obtain duplicate estimates of area (to nearest 0 .01 mm^2). Volume estimates were obtained from the means of the two area measurements using an appropriate conversion factor for the calibrated squash plate (Rakocinski and Zapfe 2005).

For common taxa (i.e., taxa represented by multiple size fractions that occur in more than 20% of the samples), general linear regressions of per individual biomass versus the size fraction were made on a log-log scale:

$$y = b(1)x + b(0)$$

where y = $\log_{10}$ of the weight per individual (g), x = $\log_{10}$ of the size fraction of the organism (mm), b(1) = the slope of the regression, and b(0) = the intercept of the regression, to enable volume estimates based on counts for any respective size fraction.

For other common large organisms, linear regressions based on a standard linear measurement of the carapace length (mm) for grass shrimp and carapace width (mm) for crabs was regressed against weight (g) for individuals on a log-log scale. Alternatively, nonlinear regressions:

$$f = a * x^b$$

where f = weight per individual (g), a = the intercept of the nonlinear regression, x = the size fraction (or measured size of the organism; mm), and b = the slope of the nonlinear regression, were used when assumptions of variance and normality failed the linear regression, the nonlinear data was homoscedastic, and/or the r^2 value was higher than the r^2 of the linear regression. Where body-size correlations were non-significant, a mean individual wet weight was used for all size fractions.

Production Estimates

Dry weights (g) were obtained from wet weights (g) using a conversion factor of 0.16 for all taxa (Ricciardi and Bourget 1998). Per individual dry weight measurements from separate size fractions were used to estimate secondary production based on the general allometric equation proposed by Edgar (1990):

$$P_{ind} = 0.0049 \times B_{ind}^{0.8} \times T^{0.89}$$

where P_{ind} = daily individual production in μg ash free dry weight (AFDW d^{-1}), B_{ind} = individual μg AFDW, and T = water temperature (°C). Temperature was standardized at 30° C based on steady temperature trends for these calculations of production potential. Daily individual production was further extrapolated into total production potential within each size fraction and total production potential for each sample, and further normalized to the area of substrate for analysis.

Community Turnover Rate

As the production to biomass ratio (P: B) reflects the period of time within which community biomass is replaced by production, the community turnover rate (days) was estimated through the inverse proportion of the P: B (Rakocinski and Zapfe 2005):

$$d = \frac{1}{(P:B)}$$

Normalized Biomass Size Spectra

Normalized Biomass Size Spectra (NBSS) were used to document differences in the distribution of biomass across various size classes. The NBSS parameters were estimated for each sample by regressing the $\log_{10}$ biomass within each size-class (standardized to the size-class range) against $\log_{10}$ values for the midpoints of the of the associated size classes (Rakocinski and Zapfe 2005). Size classes for the NBSS followed an octave size scale as defined by body mass doubling increments (Schwinghamer 1988), with the smallest size class starting at 150 µg. Parameters for linearized biomass-size spectra were estimated for regressions of cumulative total biomass per size class with respect to the size class range. Higher elevations and steeper negative slopes reflect higher abundances of small organisms and/or the lack of larger organisms, and vice versa.

Statistical Analysis

The statistical analysis to test for differences in total production (ln scale), biomass (ln scale), abundance (ln scale), and community turnover period was done using a Split-plot ANOVA. The within-subject factor for the Split-plot ANOVA was the basket substrate variable (rock or shell), to recognize their non-independence between each pair

of the baskets. The between-subjects factor was represented by reef profile type (high profile vs. low profile). In addition, the interaction between the within and between-subject factors was tested. When the sphericity assumption was not met, Greenhouse-Geisser adjusted test values were used (Greenhouse and Geisser 1959).

Diversity Metrics

Several diversity metrics were calculated for individual samples in PRIMER 6.0 (Clark and Gorley 2006) and compared across reef type (high vs low) and substrate type (rock vs shell). Shannon's diversity index (H') was calculated using following equation:

$$H' = -\sum_{i=1}^{s} (p_i)(log_2 p_i)$$

where H' is Shannon's diversity index, p_i is the proportion of the total number of organisms represented by taxon *i*, and *S* is the number of taxa (Krebs 1989). Shannon's equitability (J') was calculated by using the ratio between H' and maximum diversity (H_{max}):

$$J' = H'/H_{max}$$

Community Similarity

The community structure of the benthic macrofauna was compared between reefs based on community similarity using PRIMER 6 software (Clark and Gorley 2006). The number of organisms per sample was fourth root transformed to prevent the dominant taxa from influencing the community similarity values. Bray-Curtis community similarity values were obtained for all samples:

$$BC_{ij} = 1 - \frac{2C_{ij}}{S_i + S_j}$$

where C_{ij}=sum of the lesser values for only the species in common between both sites; Si and Sj= total number of species counted at both sites. The community similarity matrix was ordinated using Multidimensional Scaling (MDS), with groupings within the MDS ordination plot delineated via hierarchical cluster analysis using the group average cluster mode. Using SIMPER within PRIMER 6, the species contributions to differences in community similarity were examined in order to reveal faunal influences on the MDS ordination.

Nested PERMANOVA (used as an alternative to a repeated measures design within the PRIMER 6 software) tested for differences between substrate and reef profile types at an α of 0.05, based on probability distributions generated from 9999 permutations. Factors were separated into substrate type (two fixed levels), reef locations (four fixed levels), and paired basket sites (14 random levels). A separate contrast aggregated the sites into low and high profile categories. The paired basket sites were nested within the reef locations and within the profile type.

Taxon-Specific Analysis

Influential taxa, defined either by contributing to the top 20% of the mean dissimilarity between reef types in SIMPER or that were dominant in the community by contributing to the top 5% of the mean abundance, were selected for further analysis in terms of production and biomass using a Split-plot ANOVA. The within-subject factor for the Split-plot ANOVA was the basket substrate variable (rock and shell). This design recognize their non-independence between each pair of the baskets. The between-subjects factor was reef profile type (high profile vs. low profile). In addition, the interaction

between within and between-subject factors was tested. When the sphericity assumption was not met, the Greenhouse-Geisser adjusted test value was used.

For biomass and production of selected species that failed the homogeneity of variance assumption (but passed the normality assumption), responses were pooled to test for differences in profile type (differences between substrate types were nonsignificant) and were tested with one-way ANOVA, interpreted using the Welch's test (Welch 1947).

CHAPTER III - RESULTS

Abiotic Parameters

Water temperature followed the expected seasonal pattern over the six weeks post-deployment, starting with a mean temperature of 29.3°C in August followed by a steady decrease until September (Figure 3).

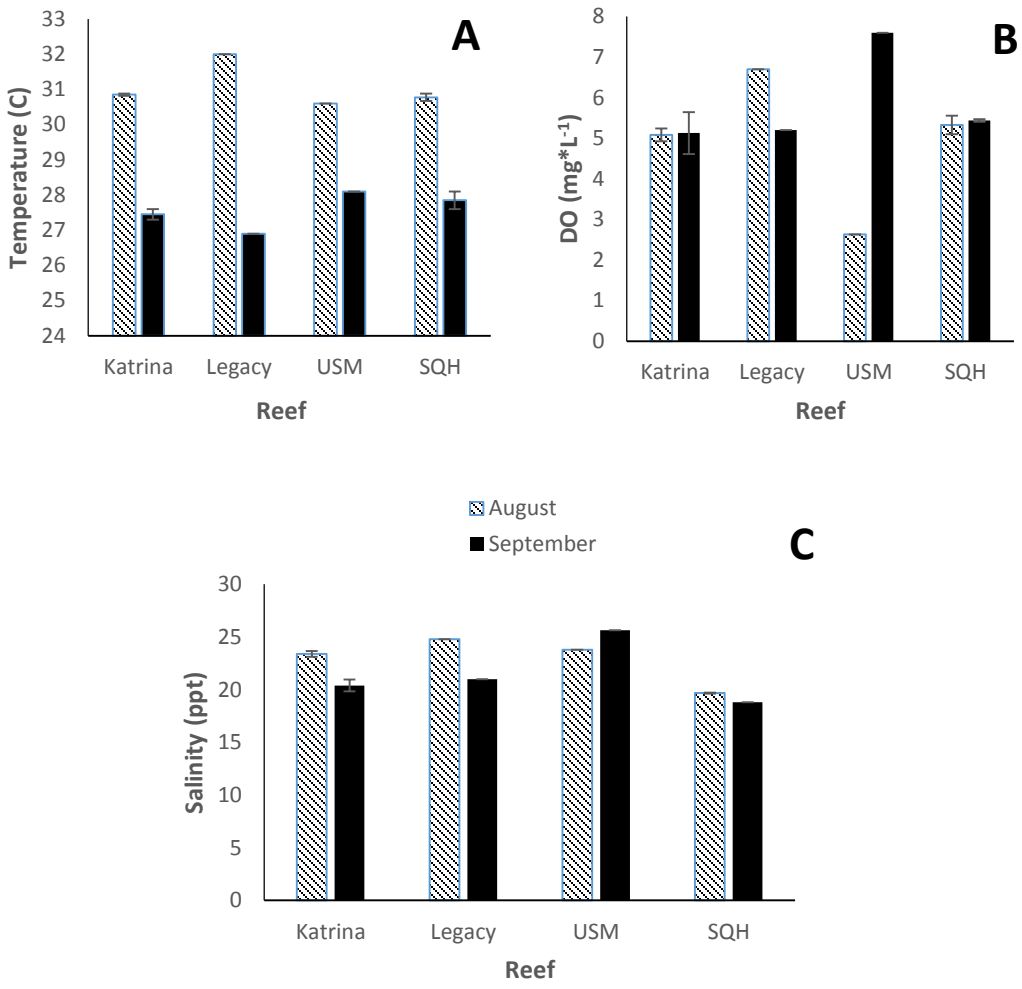

Figure 3. Mean water temperature (°C) (A), dissolved oxygen (mg x L^{-1}) (B), and salinity (ppt) (C) recorded upon deployment and retrieval of baskets at all reefs sites (n=4 for Katrina and SQH.

(± 1 SE), n=1 for Legacy and USM).

Salinity was variable among sites and dependent on pre-event rainfall, typically falling between 17 ppt to 27 ppt. Salinity seemed to be slightly higher at the low profile reefs; Square Handkerchief exhibited the lowest salinity range. Dissolved oxygen remained within similar ranges at the high profile reefs for both August and September, but no such trend was apparent at the low profile reefs.

Invertebrate Biomass, Production, and Turnover

Overall, a total of 147,077 individuals comprising 69 taxa were identified (Table 3). The most abundant taxa were polychaetes (60.3%) followed by arthropods (16.7%) and molluscs (13.1%). Polychaetes consisted primarily of *Alitta succinea* (81.3% of polychaetes) and spionids (11%); arthropods consisted primarily of amphipods (63.8% of arthropods) and panopeid crabs (24.4%); and molluscs consisted primarily of nudibranchs (58.5% of molluscs) and mussels (24.2%). Mean organism density did not differ between reef profile ($F= 0.60$, $p=0.44$) or substrate types ($F=0.01$, $p=0.08$) (Table 4). Nor did production potential (substrate- $F= 0.73$, $p=0.41$; profile- $F= 0.60$, $p=0.46$), biomass (substrate- $F= 0.23$, $p=0.64$; profile- $F= 0.46$, $p=0.51$), or the community turnover rate (substrate- $F= 0.22$, $p=0.65$; profile- $F= 0.16$, $p=0.23$) differ significantly relative to substrate or reef profile type).

Tests of assumptions for the metrics passed for normality (K-S tests; Kolmogorov 1933; Smirnov 1948) and homogeneity of variance (Levene's tests; Levene 1960). Although no significant difference was found, the power of detection was generally very low where tests failed to find differences. Still, there was considerable variability in the overall functional metrics between profile and substrate types (Figure 4).

Table 3

Mean (± 1 SD) abundances of organisms among reef sites. Square Handkerchief only had a sample size of 4 due to baskets moving off the reef.

		Mean number of organisms (m^{-2})			
Species	**Common Name**	**Katrina (n=8)**	**Legacy (n=8)**	**USM (n=8)**	**Square Hand-kerchief (n=4)**
Alitta succinea	Polychaete	1585.79 ± 1376.79	1422.57 ± 856.49	2430.59 ± 1237.12	2930.64 ± 1564.59
***Alpheus* sp.**	Snapping Shrimp	3.21 ± 3.01	0.76 ± 1.41	1.54 ± 1.54	0.88 ± 1.77
Amphilochidae	Amphipod	246.53 ± 399.90	0.85 ± 1.69	2.7 ± 5.03	0.79 ± 0.93
Amygdalum papyrium	Paper Mussel	0 ± 0	0 ± 0	0.14 ± 0.4	0 ± 0
***Ancistrosyllis* sp.**	Polychaete	0 ± 0	0 ± 0	0 ± 0	0 ± 0
***Apomatus* sp.**	Polychaete	0 ± 0	0.63 ± 1.78	0 ± 0	1.59 ± 3.18
***Astyris* sp.**	Mussel	7.19 ± 8.26	2.53 ± 4.57	0.73 ± 2.07	1.21 ± 1.5
***Autolytus* sp.**	Polychaete	7.67 ± 14.05	0 ± 0	0 ± 0	0 ± 0
***Balanus* sp.**	Barnacle	13.95 ± 26.52	4.63 ± 6.7	91.04 ± 87.79	15.06 ± 19.48
***Callinectes* sp.**	Blue Crab	2.46 ± 4.17	0 ± 0	6.66 ± 11.63	1.28 ± 1.68
***Calyptraeidae* sp.**	Gastropod	0 ± 0	0.16 ± 0.45	0 ± 0	0 ± 0
***Capitella* sp.**	Polychaete	9.68 ± 10.79	199.16 ± 149.68	34.87 ± 35.69	2.47 ± 3.08
Clibanarius vittatus	Hermit Crab	0 ± 0	3.65 ± 7.86	3.01 ± 5.14	0 ± 0
Clymenella torquata	Polychaete	0 ± 0	0.22 ± 0.62	0 ± 0	0 ± 0
Corophiide	Amphipod	278.86 ± 361.84	5.46 ± 4.57	5.52 ± 4.95	986.86 ± 650.17
Cossura delta	Polychaete	0.36 ± 1.01	0 ± 0	0 ± 0	0 ± 0

Species	Common Name	Katrina (n=8)	Legacy (n=8)	USM (n=8)	Square Handkerchief (n=4)
***Costoanachis* sp.**	Gastropod	0.16 ± 0.46	0 ± 0	0 ± 0	0 ± 0
Crassostrea virginica	Eastern Oyster	0.54 ± 1.53	16.29 ± 35.88	19.47 ± 38.56	4.09 ± 4.1
Crepidula depressa	Slipper Limpet	0.67 ± 1.41	0.22 ± 0.62	0.18 ± 0.51	0 ± 0
Diopatra cuprea	Polychaete	0.36 ± 1.02	1.18 ± 1.96	0.31 ± 0.58	0 ± 0
Glycinde solitaria	Polychaete	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Menippe adina	Stone Crab	0.67 ± 1.44	0.84 ± 0.94	3.85 ± 6.66	0.4 ± 0.8
Mytilidae	Mussel	4.52 ± 9.5	247.86 ± 169.75	162.07 ± 74.79	127.31 ± 81.95
***Nassarius* sp.**	Gastropod	0 ± 0	1.3 ± 2.86	0 ± 0	0 ± 0
Nemertea	Nemertean	35.57 ± 29.25	196.03 ± 142.75	93.85 ± 112.89	25.17 ± 14.27
***Nicon* sp.**	Polychaete	0 ± 0	0 ± 0	0.22 ± 0.63	0 ± 0
Nudibranchia	Gastropod	98.5 ± 120	441.63 ± 336.08	453.9 ± 381.87	313.38 ± 152.89
Opisthobranchia	Gastropod	3.78 ± 3.75	169.03 ± 161.51	78.71 ± 54.27	43.78 ± 29.09
***Palaemonetes* sp.**	Grass Shrimp	4.73 ± 7.86	4.18 ± 5.55	14.12 ± 6.77	16.03 ± 9.99
Panopeidae	Mud Crab	47.32 ± 28.51	95.1 ± 90.47	392.89 ± 192.89	163.98 ± 62.62
Paracaprilla tenuis	Amphipod	6.59 ± 11.31	0 ± 0	0 ± 0	0 ± 0
Parandalia americana	Polychaete	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Paraprionospio pinnata	Polychaete	0 ± 0	43.04 ± 88.55	79.15 ± 118.73	94.88 ± 148.11
***Petrolisthes* sp.**	Porcelain Crab	0 ± 0	0 ± 0	0 ± 0	0.4 ± 0.8
Podarkeopsis levifuscina	Polychaete	2.27 ± 2.5	0.58 ± 1.21	0 ± 0	0 ± 0
***Polydora* sp.**	Polychaete	38.45 ± 37.85	574.39 ± 708.48	158.16 ± 114.84	24.16 ± 12.35
Prionospio perkinsi	Polychaete	0.36 ± 1.01	0 ± 0	0 ± 0	0 ± 0
Rullierinereis mexicanus	Polychaete	4.64 ± 10.24	10.38 ± 10.19	14.32 ± 15.33	1.89 ± 1.73

Species	Common Name	Katrina (n=8)	Legacy (n=8)	USM (n=8)	Square Handkerchief (n=4)
***Sigambra* sp.**	Polychaete	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Unid. Onuphidae	Polychaete	0 ± 0	0 ± 0	0.48 ± 1.36	0 ± 0
Unid. Tubificidae	Oligochaete	0 ± 0	0 ± 0	0.73 ± 2.07	0 ± 0
Unid Anthozoa	Anemone	360.47 ± 401.96	556.73 ± 649.85	89.91 ± 136.05	29.33 ± 11.43
Unid Bivalve	Bivalve	3.73 ± 4.84	4.24 ± 6.78	16.58 ± 34.8	0.44 ± 0.88
Unid *Capitella*	Polychaete	0 ± 0	3.28 ± 9.27	0 ± 0	0 ± 0
Unid Gastropod	Gastropod	4.94 ± 8.51	1.35 ± 2.4	0.81 ± 1.86	0 ± 0
Unid Maldanidae	Polychaete	0 ± 0	3.38 ± 6.82	0 ± 0	0 ± 0
Unid Megalopa	Megalope	5.16 ± 6.11	36.04 ± 37.61	85.75 ± 67.6	8.26 ± 7.31
Unid Oligochaete	Oligochaete	0 ± 0	0.39 ± 0.72	0 ± 0	2.21 ± 4.42
Unid Ophiuroidea	Brittle Star	0.18 ± 0.51	0 ± 0	0 ± 0	0 ± 0
Unid Serpulidae	Polychaete	91.39 ± 124.12	12.94 ± 9.59	256.87 ± 337.95	5.29 ± 3.86
Unid Spionidae	Polychaete	3.36 ± 5.12	8.3 ± 13.54	12.71 ± 31.95	2.52 ± 2.92
Unid Turbellaria	Flat Worm	10.54 ± 15.01	140.04 ± 111.2	93.81 ± 66.77	51.88 ± 23.36

Table 4

Split-plot ANOVA results for total production, abundance, biomass, and turnover for (A) Within-Subject effects and (B) Between-Subject effects for Underlined refers to biologically meaningful values.

A

Dependent Variable	Source	DF	F	Sig
LnProduction	Substrate	1	0.73	0.41
	Substrate×Profile	1	0.53	0.48
LnAbundance	Substrate	1	0.01	0.94
	Substrate×Profile	1	3.63	0.08
LnBiomass	Substrate	1	0.23	0.64
	Substrate×Profile	1	0.41	0.53
Turnover	Substrate	1	0.22	0.65
	Substrate×Profile	1	0.24	0.63

B

Dependent Variable	Source	DF	F	Sig
LnProduction	Profile	1	0.60	0.46
LnAbundance	Profile	1	0.63	0.44
LnBiomass	Profile	1	0.46	0.51
Turnover	Profile	1	1.62	0.23

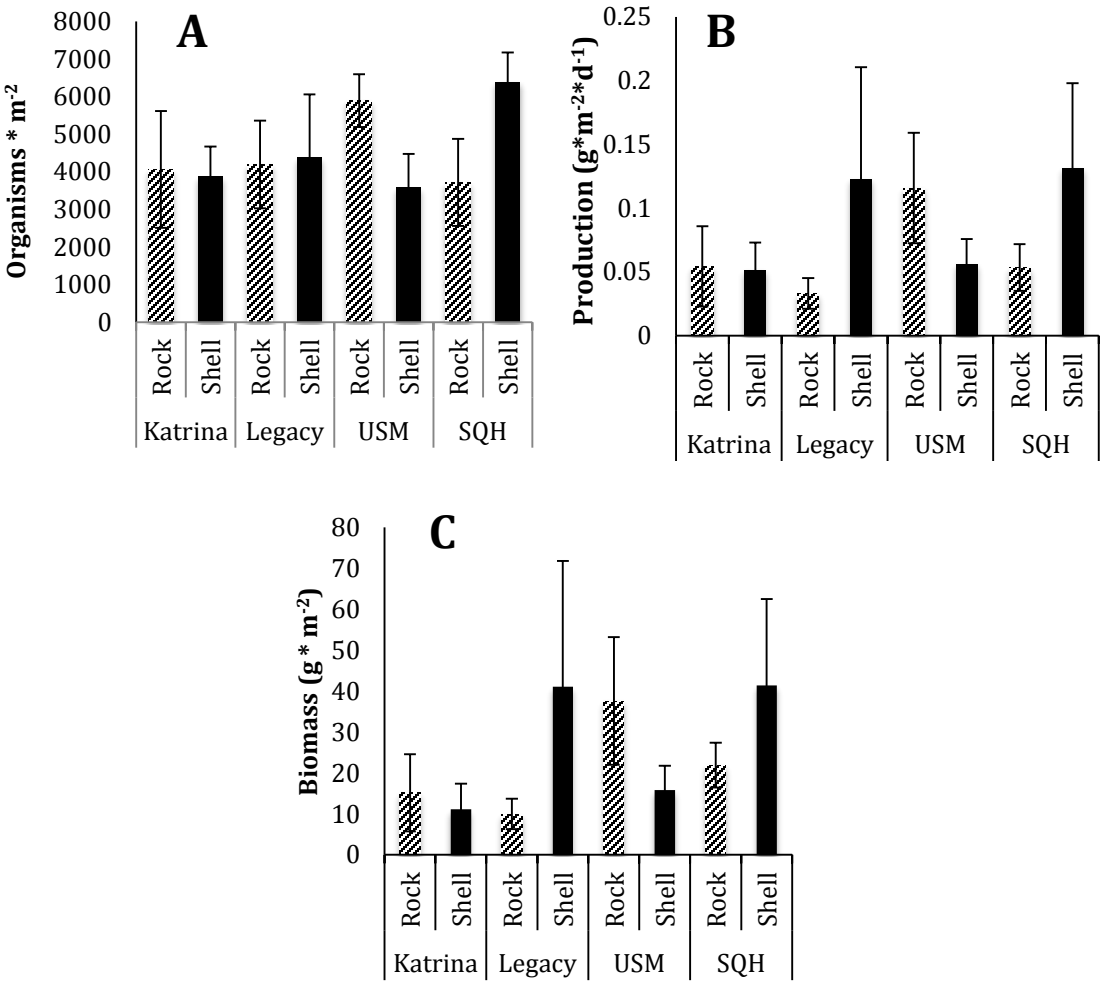

Figure 4. Mean (± 1 SE; per m^2) abundance (A), production (B), and biomass (C) between substrate (rock and shell) across all reef sites.

(Katrina, Legacy, USM and Square Handkerchief).

Size Class Structure

The bivariate relationship between slopes and intercepts of the NBSS showed a distinct difference between substrate types within the high profile reef samples (Figure 5). Shell substrate exhibited generally higher intercepts and steeper slopes than rock, implying greater biomass for small size classes on high profile rock compared to greater biomass for large size classes on high profile shell. In addition, NBSS parameters were less variable for shell than for rock from high profile reefs. Furthermore, NBSS parameter

values overlapped greatly between substrate types at low profile reefs, and spanned across intermediate ranges on both axes.

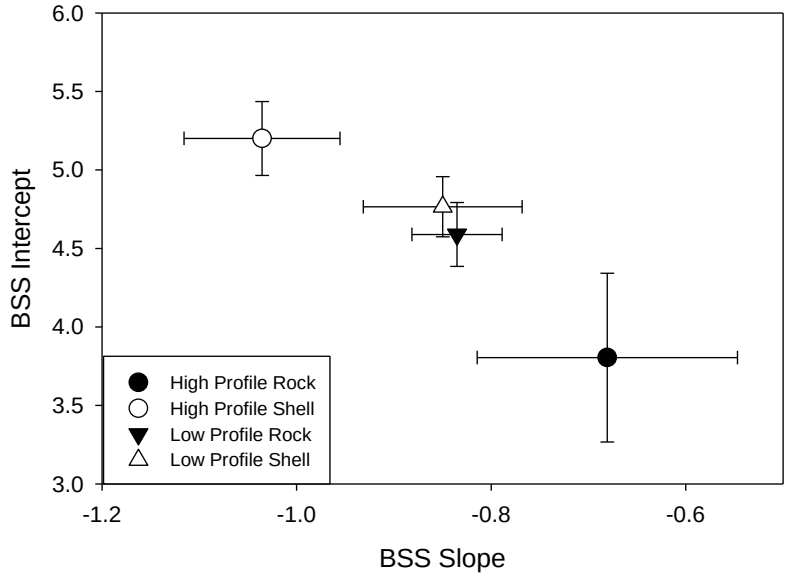

Figure 5. Normalized Biomass Size Spectra slopes vs. intercepts representing combinations of reef profile (high and low) and substrate type (rock and shell).

Diversity

There were no observed differences between substrate or reef profile types for the diversity indices examined (Table 5). Species richness did not differ much between profiles and substrates, ranging from 23.36 to 25 taxa per m^2. Equitability scores range from 0-1, with 1 representing similar abundances among taxa in the sample. For this study, evenness ranged from 0.5-0.6, reflecting that many taxa had similar abundances while some taxa dominated the sample (refer to Table 3).

Table 5

Variation in diversity x m^{-2} metrics relative to reef profile type and substrate material

	Species richness	Number of individuals* m^{-2}	SD	Equitability	SD	Shannon	SD
High Profile	24 ± 2.66	3762.91	2171.88	0.53	0.11	1.67	0.37
Low Profile	24.31 ± 2.63	4465.49	2257.46	0.60	0.07	1.91	0.22
Rock	23.36 ± 2.37	4256.88	2209.42	0.59	0.07	1.86	0.25
Shell	25 ± 2.63	4071.89	2287.34	0.55	0.11	1.76	0.37

Values are means ± 1 SD.

Community Structure

MDS ordination depicted variation in the community structure among individual samples based on faunal abundances (Figure 6). The hierarchal cluster analysis showed that all samples fell within a margin of 40% similarity. The 2D stress of 0.16 for the MDS indicated that community similarity was fairly well represented within the 2D plot (i.e., <0.2). Invertebrate samples from Legacy Reef, USM Reef, and Square Handkerchief Reef all fell within a 60% similarity envelope. Additionally, all Katrina Reef samples fell within a 60% similarity envelope with the exception of one anomaly. The lower profile reefs, USM and Legacy, were 70% similar, but they showed some overlap between samples. Katrina reef showed the greatest variability in community structure and the least similarity with the other reefs. Apparent non-independence of pairs of baskets was shown by a pattern of close proximity within the MDS, as seen most strongly for Katrina reef.

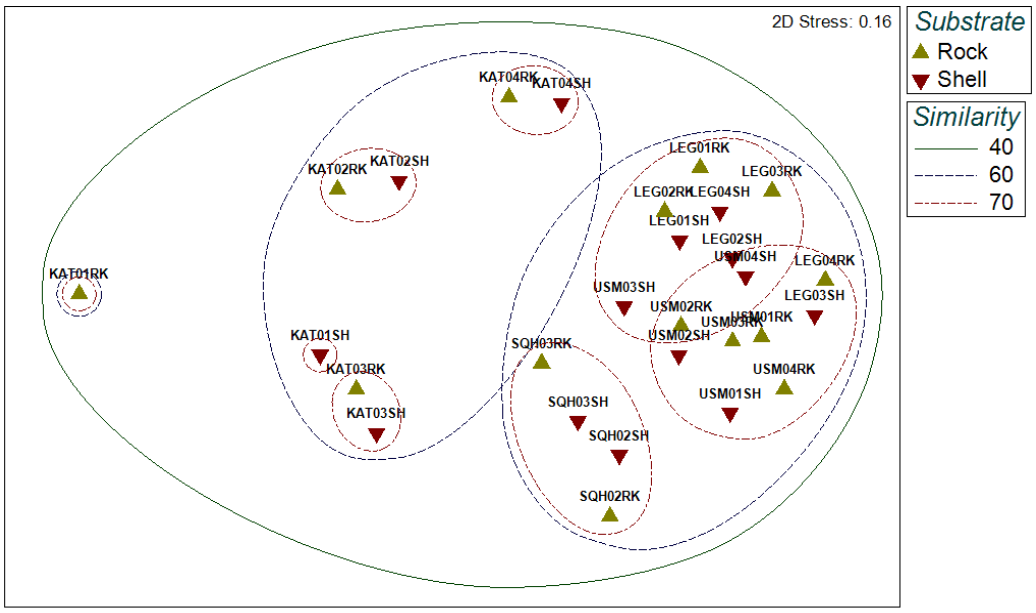

Figure 6. Ordination plot within the first two MDS dimensions, showing macrofaunal community similarity between substrate and reef profile types. Similarity groupings (ovals) based on hierarchical cluster analysis using the group average cluster mode.

SIMPER identified the taxa that contributed most to explaining similarity in community structure. The mean similarity within high profile reefs was 62.60% and 71.57% within low profile reefs. A mean dissimilarity of 41.16% was seen between the two reef profile types. *Alitta succinea* (formerly *Neanthes*) (Villalobos-Guerrero and Carrera-Parra, 2015) dominated the community structure on both high and low profile reefs. Various amphipod taxa (corophiids, melitids, amphilochids), mytilid mussels, and polychaetes (capitellids and *Polydora*) mainly contributed to community dissimilarity between the high and low profile types (Table 6).

The mean similarity within rock substrate was 62.07%, and within shell substrate it was 64.31%; whereas the mean dissimilarity between the two substrate types was 36.14%. Many of the same species drove community dissimilarity for both substrate

Table 6

Mean dissimilarities between reef profile type (high and low) (A) and substrate type (rock and shell) (B) for artificial reefs in the Mississippi Sound using SIMPER analysis for top 20% contributing taxa

A

Taxon	High Profile Abundance	Low Profile Abundance	Mean Dissimilarity
Corophiidae	4.09	1.26	2.33
Melita	2.91	0.55	1.97
Mytilidae	1.59	3.66	1.81
Amphilochidae	2.25	0.39	1.76

B

Taxon	Rock Abundance	Shell Abundance	Mean Dissimilarity
Corophiidae	2.52	2.42	1.61
Amphilochidae	1.33	1.04	1.35
Mytilidae	2.67	2.88	1.30
Melita	1.50	1.62	1.28
Paraprionospio	1.27	0.97	1.28

types, including *Alitta succinea*, nudibranchs, panopeid crabs, anthozoans, and *Polydora*. Dissimilarity between substrate types was driven by various amphipods (corophiids, amphilochids, *Melita*), as well as mytilids and the opportunistic polychaete, *Paraprionospio pinnata* (Table 6).

PERMANOVA supported the patterns in the MDS of Bray Curtis similarity values (Table 7). Significant differences were found among reef locations (F=1.94; P=0.001), with the Katrina Reef assemblage showing the most dissimilarity with the other three assemblages (Figure 7). Significant differences were also observed between profile types (F=5.88; P=0.001) and for samplers nested within profiles (F=2.56; P=0.001). The low profile reefs showed similar community patterns while the high profile reefs showed slightly less similar community similarity. Although community similarity of paired baskets visually grouped within MDS space, assemblages were not different between substrate types (F=1.10; P=0.37), or in terms of the substrate by reef interaction (F=0.93; P=0.59) or profile (F=1.34; P=0.26).

Table 7

PERMANOVA of Bray-Curtis similarity values relative to substrate, profile, and reef for invertebrate samples.

Source	**Df**	**SS**	**MS**	**Pseudo-F**	**P**
Substrate	1	360.19	360.19	1.10	0.37
Site	3	7986.10	2662.00	4.19	**0.001**
Profile	1	4712.00	4712.00	5.88	**0.001**
Station(Site)	10	6348.30	634.83	1.94	**0.001**
Station(Profile)	12	9622.30	801.86	2.56	**0.001**
Substrate × Site	3	909.630	303.21	0.93	0.59
Substrate × Profile	1	420.47	420.47	1.34	0.26
Residual	10	3274.00	327.40		
Total	27				

Taxon-Specific Responses

The substrate type effect was non-significant for all taxa examined (see Table 8 for summary of statistics); however, biologically meaningful effects of substrate type (i.e., $P < 0.09$) for the polychaete *Alitta succinea* showed marginally higher biomass on the shell substrate. In contrast, nudibranch production had biologically meaningfully higher production values for rock on high profile reefs. Significantly greater production occurred on high profile reefs for amphipods in the families Corophiidae ($F=41.23$, $P<0.001$), Amphilochidae ($F=6.69$, $P<0.05$), and Melitidae ($F=45.03$, $p<0.001$). Conversely, the production of Mytilidae (mussels) was significantly greater on low profile reefs ($F=9.97$, $P<0.01$). The only taxon showing a biologically meaningful interaction between substrate and profile was Melitidae, whose biomass ($F=3.499$, $p=0.09$) was greatest on shell of low profile reefs. Although the variance assumption was violated within the original Split-plot ANOVA for Corophiidae, Mytilidae, Amphilocidae, and Melitidae, further analysis as a one-way ANOVA using Welch's test confirmed significant statistical differences between reef profile types for these taxa (Table 9).

Table 8

Summary table of taxon-specific differences between reef profile type (high and low) and substrate types (rock and shell) among four artificial reefs in the Mississippi Sound

	Variable	Effect	F	Significant	Direction	Welch's
Alitta succinea	Biomass	Profile	1.93	None	Low	NA
		Substrate	3.49	0.09	Shell	
		Interaction	0.98	None		
	Production	Profile	1.60	None	Low	NA
		Substrate	3.52	None	Shell	
		Interaction	1.30	None		
Panopeidae	Biomass	Profile	1.23	None	High	NA
		Substrate	1.47	None	Rock	
		Interaction	0.83	None		
	Production	Profile	0.47	None	High	NA
		Substrate	0.07	None	Interaction	
		Interaction	1.16	None		
Corophiidae	Biomass	Profile	7.48	**P<0.05***	High	0.07
		Substrate	0.07	None	Equal	
		Interaction	0.10	None		
	Production	Profile	41.23	**P<0.001**	High	NA
		Substrate	0.52	None	Equal	
		Interaction	1.37	None		

Mytilidae	Biomass	Profile	0.05	None	Low	NA
		Substrate	0.19	None	Equal	
		Interaction	0.00	None		
	Production	Profile	9.97	**P<0.01***	Low	**0.032**
		Substrate	0.32	None	Equal	
		Interaction	0.001	None		
Nudibranch	Biomass	Profile	2.82	None	Low	NA
		Substrate	2.75	None	Interaction	
		Interaction	2.21	None		
	Production	Profile	3.04	None	Low	NA
		Substrate	3.49	0.09	Interaction	
		Interaction	3.18	None		
Amphilochidae	Biomass	Profile	3.77	0.08*	High	0.16
		Substrate	2.46	None	Interaction	
		Interaction	2.50	None		
	Production	Profile	6.69	**P<0.05***	High	**0.05**
		Substrate	1.18	None	Interaction	
		Interaction	1.83	None		
Melitidae	Biomass	Profile	7.99	**P<0.05***	High	**0.03**
		Substrate	3.33	None	Interaction	
		Interaction	3.50	None		
	Production	Profile	45.03	**P<0.001**	High	NA
		Substrate	0.32	None	Equal	
		Interaction	0.69	0.09		

*Heterogeneous variance; Bold refers to significant values; Underlined refers to biologically meaningful values)

Table 9

Welch's Test results for Mytilidae and Amphilochidae production and Corophiidae, Amphilochidae, and Melita *biomass between reef profile types. Bold refers to significant values; Underlined refers to biologically meaningful values.*

	F	**df1**	**df2**	**Sig.**
Mytilidae Production	7.81	1	5.928	**0.03**
Amphilochidae production	5.49	1	6.698	**0.05**
Corophiidae Biomass	5.46	1	5.001	<u>0.07</u>
Amphilochidae Biomass	2.75	1	5.013	0.16
***Melita* Biomass**	8.33	1	5.041	**0.03**

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CHAPTER IV – DISCUSSION

Functional Metrics

Both rock and shell substrates have proved to be successful materials for the recruitment of benthic organisms in a number of studies (Baine 2001; Menge and Sutherland 1987; Grabowski 2004). Typically, high profile reefs are designed to attract pelagic fish species, while low profile reefs with heterogeneous void spaces are a more effective shellfish attractant (Baine 2001). Additionally, increased substrate complexity fosters benthic production (Markert *et al.* 2010). However, in this study, overall functional metrics for the entire benthic invertebrate community did not differ between substrates or reef profile types, although taxon-specific differences in production and biomass were apparent between reef profile types. Markert *et al.* (2010) noted a difference in the functional feeding group of polychaetes associated with reef complexity. Reefs with more complexity (i.e., full oyster reefs) were dominated by surface deposit and suspension feeders, while less complex reefs were only dominated by deposit feeders. While this distinction was not made clear in the present study, multiple species of deposit feeding polychaetes (*Capitella*, *Polydora*) and several species of both deposit and suspension feeding molluscs (mytilds, opisthobranchs, nudibranchs) occurred in much higher abundances on the low profile reefs than the high profile reefs, showing a striking similarity to the functional feeding groups found in the study by Markert *et al.* (2010).

Adjacent habitats can play a major role in regulating artificial reef assemblages by mediating both the quality and quantity of food resources (Bohnsack *et al.* 1991; Randall 1963). High profile reefs in my study rest on top of mostly anoxic mud, which may

prevent certain organisms from settling, because surrounding sediment cannot serve as a source of recruitment. The surrounding sediments of reefs undoubtedly mediate in the abundances of some taxa. Capitellids are found in a variety of sediment types, but predominantly in organically rich sediments (Uebelacker and Johnson 1984); whereas, species of *Polydora* often form tubes using a variety of materials including sand, but may also found boring into both living a dead shell (Reish 1968; Uebelacker and Johnson 1984). It is likely that reef construction material could also affect recruitment (Markert *et al.* 2010). Different base substrates like sand and shell may have elicited differences in abundances of certain grazers, deposit feeders, and predators, including opisthobranchs, nudibranchs, nermeteans, and turbellarians, which occurred in much higher abundances on the low profile reefs. The presence of these organisms also suggests that benthic primary production also plays a major role in the food web of artificial reefs (Cresson *et al.*, 2014). Mazzei and Biber (2015) confirmed that benthic primary production was greater at low profile reefs than high profile reefs during this sampling period, which also corresponds with differences in depth and basal substrate.

Colonization by certain fouling taxa may differentially enhance the surface area complexity between reef profile types. For example, colonization by zebra mussels, *Dreissena polymorpha*, increases interstitial space and benthic organic matter concentrations, leading to more benthic biomass (Stewart *et al.* 1998). It is likely fouling taxa also played a role in augmenting the habitat complexity of the MS artificial reefs. Mussels, which occurred in much greater abundances at the low profile reefs, can potentially increase overall surface area and habitat complexity, which may favor further recruitment. Although mussels alone were inferior to oyster reefs in terms of promoting

recruitment (Markert *et al.* 2010), their presence within oyster reefs may enhance recruitment. Additionally, the increased presence of filter feeders suggests a food from a pelagic source has a dominant role in the food web (Cresson *et al.* 2014). A pelagic food source was confirmed as a major component of the reef food web in the MS Sound by Mazzei and Biber (2015). Further, the increased occurrence of amphipods is consistent with increased bryozoan biofouling (Martin and Bortone 1997). Salamone (2012) noted a high prevalence of biofouling on the same high profile reefs as this study, which corresponds with the relatively high abundance of amphipods. Peracarid crustaceans (primarily amphipods) (e.g., corophiids, *Melita*, amphilochids, *Grandidierella*, *Hargeria*) occurred either exclusively or in much higher abundances on the high profile reefs compared to the low profile reefs.

In my study, polychaetes (primarily *Alitta succinea*) largely occurred inside of oyster shell crevices, suggesting complex structural matrix (personal observation) favored high abundances of *Alitta succinea*. Markert *et al.* (2010) also showed that differences in the geometry of oyster shells creates high interstitial space and vertical relief, which could also help explain why *Alitta succinea* occurred on low profile reefs in high abundance. In Markert *et al.* (2010), differences between oyster and mussel reef types accounted for twice the total abundance and biomass of benthic organisms in oyster compared to mussel beds, may also reflect the influence of boring on benthic abundance and biomass. Crustacean abundances have previously been shown to be higher in reef habitats compared to non-structured habitats (Xu *et al.* 2014). Further, relatively high abundances of amphipods suggest a greater development of fouling communities on high profile reefs than on low profile reefs. Both *Melita nitida* and *Hargeria rapax* were either

highly abundant or exclusively occurred on high profile reefs, and both taxa are typically associated with fouling communities (Hoese *et al.* 1972; Modlin and Harris 1989; LeCroy, 2000).

Conversely, certain taxa were more abundant on low profile reefs than on high profile reefs. For example, although mobile mud crabs occurred everywhere, they were more abundant on low profile reefs. Glancy *et al.* (2003) showed that oyster reefs harbored a 15-fold greater abundance of mud crabs compared to marsh edge (considered bare) and two-fold greater than that of seagrass. These differences strongly related to the greater coverage, interstitial space, and the three-dimensional structure of oyster reefs. Mud crabs often associate with hard substrates (most commonly oyster reefs), and their distribution is often limited by salinity (May 1974). Ryan (1956) showed a positive correlation between *Eurypanopeus depressus* and the presence of dead and live oyster shell in the Chesapeake Bay. *Eurypanopeus depressus* can tolerate much lower salinities than *Panopeus herbstii* (May 1974). However, *P. herbstii* and *E. depresssus* share the same feeding niche and occupy similar microhabitats among small spaces within dead and living oyster shells (McDonald 1982). Interestingly, *Panopeus obseus* did not occur in this study, although this is a common mud crab species in the MS Sound. This species often co-occurs with *P. herbstii*, from which it is vertically segregated. *Panopeus obseus* tends to build burrows under live oyster shell; whereas, *P. herbstii* instead hides within shelter created by shell (Menendez 1987). This suggests that some taxa may have been inadvertently excluded by sampling bias associated with the use of substrate baskets. The inability to build an appropriate burrow under the shell may have precluded the collection of *P. obseus*.

Comparisons with Other Habitats

The analysis of benthic samples collected using a Van Veen grab from estuaries of the northern Gulf of Mexico exhibited a mean biomass of 7.06 g m^{-2} and a density of 2,846.4 organisms m^{-2} (Gaston *et al.* 1997). These values are much lower than the mean biomass of 24.56 ± 13.26 g m^{-2} and the mean density of 4,296.9 ± 884.58 org m^{-2} seen within the MS artificial reefs as found in my study. The bulk of the taxa in the northern Gulf of Mexico samples were suspension feeding molluscs. Typically, benthic faunal biomass is higher in the structured habitats than on mudflat (Castel *et al.* 1989; Hosack *et al.* 2006). Hosack *et al.* (2006) also found that densities and community composition of benthic organisms were significantly different between adjacent patches of eel grass, oyster reef, and mudflat. Generally, studies have found relatively higher densities, biomass, and species richness with such structured habitats (Coen *et al.* 2007).

Biomass varies greatly across a variety of habitats within North Carolina (NC), including natural shorelines and man-made structures (Wong *et al.* 2011). Biomass varied widely between 0.220 and 713.5 g AFDW x m^{-2} across intertidal and subtidal flats, bulkheads, marsh, seagrass, and oyster reef. Once more, biomass at structured habitats was consistently higher than unstructured habitat. This range of values compared to my study with a mean biomass of 3.38 ± 1.92 g AFDW m^{-2} for the rock sampler and 4.47 ± 2.45 g AFDW m^{-2} for the shell samplers found in the MS artificial reefs showing that these reefs have a much more biomass present in structured environments than the unstructured environments of NC. However, even within the structured environments (i.e, seagrasses), biomass varied greatly among sites. Production potential similarly varied greatly among structured habitats, particularly oyster reefs at Middle Marsh, NC, which

were dramatically higher than other habitats even excluding large molluscs (160 g $m^{-2}y^{-1}$) (Figure 7).

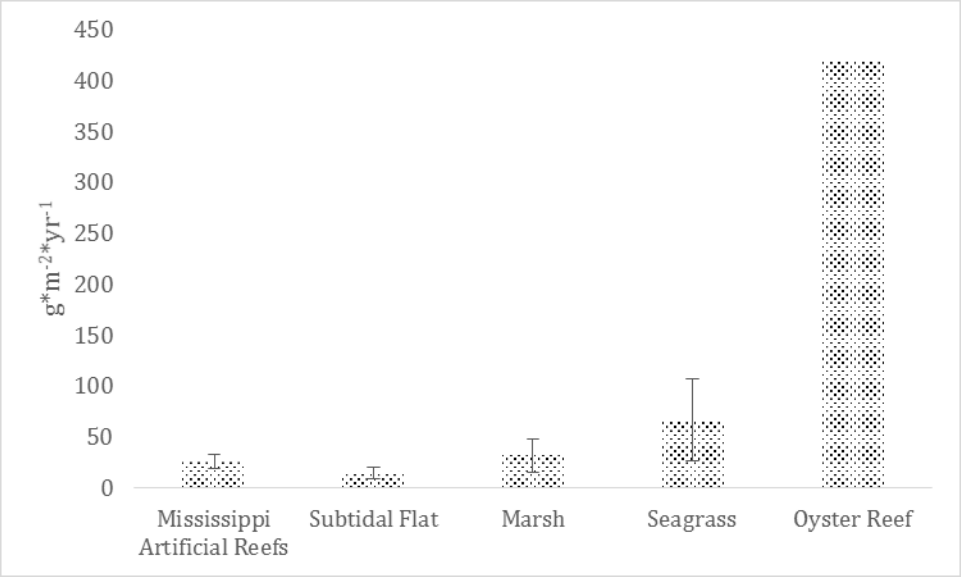

Figure 7. Production potential on Mississippi artificial reefs from this study and for a variety of habitats in North Carolina estuaries.

Wong et al. 2011.

Peterson *et al.* (2008) also observed secondary production of oyster reefs that was 20.7 times higher than seagrasses, and 21.8 times higher than subtidal mudflats in NC.

Reef Sites

The effect of complexity is likely taxon-specific, depending on life history traits and habitat requirements. However, the positioning, construction, and configuration of the reef sites must also be considered. Aside from bottom topography and sediment properties, the reef settings in my study differed in other potentially confounding ways. Both high profile reefs are located considerably further offshore than the low profile reefs, and both high profile reefs are much closer to riverine output than the low profile reefs (Barnes 2014). These differences in turn likely relate to additional variability in

abiotic factors, such as salinity and temperature. Temperature fluctuations are greater near shore, which may in turn influence species occurrences (Woods, 1999). While temperatures appear consistent among reefs throughout the year, salinities are much more variable (Mazzei and Biber 2015). Woods (1999) states that although salinity often determines what species recruit to a reef, offshore placement of a reef where salinity is more stable would be more effective for recruiting biomass. Although many estuarine species are euryhaline, variability in ambient physical conditions may hinder more stenohaline species from thriving at these reefs. Abiotic data during my study showed generally lower salinities at Square Handkerchief Reef, most likely due to the river proximity. This could have had an effect on the reef community; however, the majority of species examined in my study are euryhaline and as such may not be influenced by salinity as much as by the riverine detritus output. Inordinate rainfall compelled the state of Louisiana to open the Bonnet-Carre spillway for 42 days, beginning on May 15, 2011, shunting huge amounts of freshwater discharge into Mississippi Sound (http://www.-mvn.usace.army.mil/Missions/MississippiRiverFloodControl/BonnetCarreSpillwayOverview/SpillwayOperationInformation.ax). While salinity was normal during sampling, the possibility that this event may have affected one or more reefs before sampling cannot be ruled out.

Another construction design effect of high profile reefs involves their configuration relative to wave exposure, as it affects the benthic community (Woods 1999). The high profile reefs both experienced turbulent wind, wave action, and currents because of their increased distances from shore. Russell (1975) found that wave action was the single most important factor affecting an artificial reef community; it limited

both succession and colonization of the benthic community. Because of the orientation of Katrina reef, wave action was a source of turbulence on its south side, whereas the north side was largely protected from wave action. This may have been a factor affecting the broad spread of the four sets of paired samplers at Katrina Reef in the MDS ordination space when compared to the other three reefs. On the other hand, neither side of Square Handkerchief was consistently protected or disturbed; exposure to strong wave action varied with the wind direction. In a study by Branch and Odendaal (2003), wave action had a detrimental impact on limpet populations. In protected areas, limpets were 65% larger, comprised 80% greater biomass, included a 40% higher proportion of females, and showed 25% greater survivorship, and 33% greater growth. Woods (1999) cautioned that reefs not be deployed in areas of continuous wave action or excessive currents. Disturbance from wave action and currents may hinder the succession of the reef community by preventing the community from reaching a stable and mature stage of succession. Boat traffic was also much greater at the high profile sites (personal observation), suggesting these sites were also disturbed by heavy recreational fishing, boat wakes, etc. Likewise, basket samplers may have been subject to some manual displacement at high profile reefs, since the buoys may easily be confused with crab pots. Certain baskets (i.e., KAT01RK) were partially submerged in mud, while still showing successful recruitment on the exposed material. However, they also appeared to be an outlier within the MDS, showing how surrounding mud bottom may influence recruitment on hard substrate at high profile reefs.

General Assessment of Concurrent MS Artificial Reef Studies

This study was part of a larger ecosystem-based project supported by the Coastal Impact Assistance Program (CIAP) that examined multiple functional aspects of the same reefs examined in this study, including biofilms, primary production, and benthic and pelagic fish communities. Wilking (2013) found microbial biofilms were much better developed on low than on high profile reefs, as confirmed by higher abundances of biofouling macrofauna consisting primarily of barnacles. This also agrees with data from my study, in that another sedentary suspension feeder, mussels, occurred in higher abundances on low profile reefs. Conversely, Salamone (2012) also found major differences in the community composition of fungal biofilms between all four reefs, but in her study the high profile reef Square Handkerchief exhibited a noticeably thicker layer of biofilm. The occurrence of better developed biofilms on Square Handkerchief observed by Salamone (2012) may compliment the macrofaunal community structure observed in my study, given more amphipods occurred on high profile reefs. Kobak *et al.* (2013) found that gammarid amphipod abundances were greatly increased by shells covered in biofilm, and Tank and Winterbourne (1995) demonstrated biofilms as an important food source for grazing amphipods. Biofilms may play an additional role in promoting invertebrate communities by mediating which invertebrates can settle (Rahim *et al.* 2004). As such, biofilms play a major role in the microbial community, particularly with respect to the processing of detrital material (Salamone 2012). The biofilms may also be a major carbon source in these environments, predominantly for grazers and deposit feeding organisms

Mazzei and Biber (2015) showed net plankton and benthic productivity were both significantly higher at or on the high profile reefs, suggesting food webs of high profile reefs were most likely driven by phytoplankton derived carbon. While primary productivity was greatest in the summer, suspended particulate organic matter was greater in the fall and winter. Thus, it is likely that detrital input from nearby marshes can play a major role in driving these systems. These two alternative carbon sources can be related to my study through the feeding mechanisms of the benthic organisms. Many benthic species are opportunistic feeders and are capable of ingestion through various mechanisms (Marsh and Tenore 1990). Omnivorous polychaetes (primarily *Alitta succinea*) may be deposit feeders, predators, or scavengers. Mud crabs can be deposit feeders or predators. Amphipods (primarily corophiids and *Melita*) may be deposit or suspension feeders. Other benthic suspension feeders include serpulids, bivalves, and anthozoans. Examples of these feeding strategies were found at all reef sites in varying amounts. Deposit feeders and scavengers were particularly abundant at all reefs; however, dominant suspension feeders were more variable among reefs. Secondary production was primarily driven by panopeid crabs and nereid polychaetes at all sites. However, to a lesser extent amphipods contributed considerably to secondary production at the high profile reefs, whereas mytilids and other polychaetes contributed more to production at the low profile reefs. Stable isotope studies on these organisms from these reef sites would help distinguish the carbon sources that are driving these systems.

Matten (2013) found that reef-associated resident cryptic fish assemblages on the same reefs as those studied here were patchy and variable among reefs. However, resident fishes were significantly more abundant on low profile reefs, possibly due to reef

complexity. Diets of the benthic Skilletfish, *Gobiesox strumosus*, included a broad range of invertebrates, but were primarily made up of xanthid crabs. The predominance of mud crabs on low profile reefs seen from the present study agrees with Matten (2013), and illustrates how mud crabs provide an essential food item on the artificial reefs, especially on low profile reefs. Yeager and Layman (2011) provide further evidence that mud crabs make up an essential part of the trophic web of oyster reefs, as they occurred in the diets of Gray Snapper (*Lutjanus griseus*), Oyster Toadfish (*Opsanus tau*), and stone crabs (*Menippe adina*).

Barnes (2014) found no significant differences in the catch-per-unit-effort of transient Sand Seatrout, *Cynoscion arenarius* and Spotted Seatrout, *Cynoscion nebulosus*, among the same reefs. As these two fishes are opportunistic feeders, reef type does not appear to provide them with differential feeding benefits. Accordingly, Barnes (2014) showed that the diet composition of both species primarily consisted of fish, shrimp, and crabs across the four reefs. This further extends the notion of the trophic importance of the macrobenthos, which provides food for recreational and commercially important transient species as well. However, additional studies on benthic-feeding fish are necessary in order to connect the trophic links between benthic and pelagic communities on artificial reefs.

Conclusions

As Glancy *et al.* (2008) states, "Epifaunal invertebrates have the ability to choose among habitats with different qualities that lead to enhanced growth and survival, and as such, habitats with higher abundances are assumed to be more valuable." The four artificial reefs examined in this study represent areas of increased secondary production,

and so can be considered "valuable," particularly since the reef benthos comprises important links in the food web. My study referred to the same key functional groups as described by others (e.g., the variety of deposit and suspension feeding polychaetes found on oyster beds). The predominance of nereids and mud crabs is also consistent with other studies. Although no significant differences were found in total production, biomass, or abundance between substrate or reef profile types, differences were seen in taxon-specific production and biomass for both reef profile types. Moreover, community structure was significantly different between reef profile types. While overall patterns were not very different between substrates, biomass size-spectra did differ between rock and shell at the high profile reefs. An additional season spring/summer set of samples will lend further insights into factors influencing secondary production and diversity on these reefs. Perry *et al.* (2001) found large seasonal differences in macrobenthic invertebrates, so some differences are expected.

In terms of reef management, the creation of low profile reefs may be a more effective strategy for increasing secondary production as a food resource for higher trophic levels. High profile reefs require large amounts of source materials, heavy equipment, and manpower compared to the low profile reefs. In terms of the food web, mud crabs make up an important part of the diets for multiple recreational and commercially important species. While production potential of mud crabs seemed to be higher on high profile reefs, their abundances were higher on the low profile reefs, probably reflecting greater abundances of small crabs at the latter sites (particularly USM reef). These results agree with those of Matten (2013), indicating that the low profile reefs were more suitable for numerous small organisms owing to a higher availability of

appropriate sized refuges. Designing an intermediate type structure of more increased complexity might help maximize production. Since, no strong differences were seen relative to the type of substrate material (especially on the low profile reefs), continuing the deployment of shell would appear to be a reasonable strategy. However, peak production of artificial reefs depends on a complex interaction of multiple factors. Considerable research is still needed to determine how to maximize production yield from artificial reefs. Such future studies could also be tailored to specific target taxa, depending on the management goals.

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